CLAIMS

1. A method for detecting PrP in a biological sample of human or animal origin that may contain said PrP, characterized in that it uses a molecule having at least one positive charge and/or at least one glycosidic bond and a ligand other than a protein ligand chosen from macrocyclic and glycosaminoglycan ligands.

2. The method for detecting PrP as claimed in claim 1, characterized in that said molecule having at least one positive charge and/or at least one glycosidic bond is added to said biological sample so as to precipitate the PrP, before the addition of the ligand other than a protein ligand.

3. The method for detecting PrP as claimed in either of claims 1 and 2, characterized in that it comprises the additional step of adding proteinase K to the sample.

4. The method for detecting PrP as claimed in any one of claims 1 to 3, characterized in that it comprises the steps consisting in:

a) adding proteinase K to said sample so as to digest the PrP^c,

b) adding, to the mixture thus obtained, said molecule having at least one positive charge and/or at least one glycosidic bond so as to obtain PrP aggregates,

c) adding a ligand other than a protein ligand, and

d) revealing the presence of PrP^{res}.

5. The method for detecting PrP as claimed in any one of claims 1 to

4, characterized in that it comprises at least one of the following additional steps i) and ii), consisting in:

i) separating the PrP aggregates from the reaction mixture, and

ii) denaturing the PrP aggregates,

these steps being included, where appropriate, between step b) and step c).

6. The method for detecting PrP as claimed in any one of claims 1 to 5, characterized in that a PrP-specific binding partner for an immunoreaction between the PrP-specific binding partner and the PrP is added, where appropriate in step d).

7. The method for detecting PrP as claimed in any one of claims 1 to 6, characterized in that the ligand other than a protein ligand is bound to a solid support.

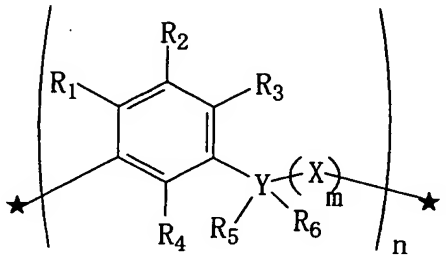
8. The method for detecting PrP as claimed in any one of claims 1 to 7, characterized in that the molecule having at least one positive charge and/or at least one glycosidic bond is a molecule having at least two guanidinium and/or ammonium functions, preferably streptomycin, more preferably in the form of a salt.

9. The method for detecting PrP as claimed in any one of claims 1 to 8, characterized in that the ligand other than a protein ligand is a macrocyclic ligand.

10. The method for detecting PrP as claimed in claim 9,

characterized in that the macrocyclic ligand is chosen from metacyclophanes, preferably from calixarenes.

11. The method for detecting PrP as claimed in claim 10, characterized in that the macrocyclic ligand corresponds to general formula (I) below:



(I)

in which

R_1 represents a hydrogen atom, a hydroxyl group, an OR group or an OCOR group, R being as defined below,

R_2 represents a hydrogen atom or an R, COR, Pol or CH_2Pol group, in which Pol represents a phosphate, sulfate, amine, ammonium or carboxylic acid group, and R is as defined below,

R_3 represents a hydrogen atom, a hydroxyl group, an OR group or an OCOR group in which R is as defined below,

R_4 represents a hydrogen atom, a hydroxyl group, an OR group, an OCH_2R group or an OCOR group, in which R is as defined below,

Y is a carbon, nitrogen or sulfur atom,

R_5 and R_6, each independently, are absent or represent a hydrogen atom, a CH_2 group or an R group as defined below, or else

R_5 and R_6 together represent an oxygen or sulfur atom,

X represents a CH_2 group, or an oxygen or sulfur atom,

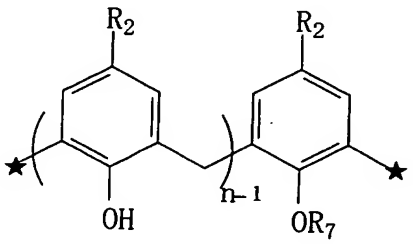
m represents an integer equal to 0 or 1,

R represents a hydrogen atom or a saturated or unsaturated, branched or unbranched, cyclic or noncyclic hydrocarbon-based chain which may or may not be substituted with a halogen group, and which carries polar or nonpolar functions,

n is an integer between 3 and 15,

the substituents R_1 to R_5, R, X and Y and the integer m may be different in nature according to the units.

12. The method for detecting PrP as claimed in claim 11, characterized in that the macrocyclic ligand corresponds to general formula (Ia) below:



(Ia)

in which

n is an integer between 4 and 8,

each group R_2, taken independently, is a sulfate group or a phosphate group,

R_7 represents a $(CH_2)_t$-$(CO)_s$-(NH_2) group or a $(CH_2)_t$-COOH group where t is an integer between 0 and 6 and s is an integer between 0 and 6.

13. The method as claimed in claim 12, characterized in that said ligand is a calixarene of formula (Ia) in which the two groups R_2 are each a sulfate group, n is 4, 6 or 8, and R_7 is a hydrogen atom, a -CH_2COOH group, a -CH_2CONH_2 group or a -$CH_2CH_2NH_2$ group.

14. The method for detecting PrP as claimed in claim 10, characterized in that the macrocyclic ligand corresponds to general formula (Ia) in which n = 6, X = Y = sulfate and R_7 is $-CH_2CH_2NH_2$.

15. A method for detecting PrP, in particular PrP^{sc}, characterized in that it comprises the step consisting in bringing a biological sample, derived or obtained from an animal or human organism, into contact with a molecule having at least two guanidinium and/or ammonium functions, with the exception of the antibiotics.

16. A diagnostic kit for detecting PrP, characterized in that it comprises a ligand other than a protein ligand chosen from macrocyclic ligands and glycosaminoglycans, and a molecule having at least one positive charge and/or at least one glycosidic bond.

17. The diagnostic kit as claimed in claim 16, characterized in that said ligand other than a protein ligand is bound to a solid support.

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Title

Method for detecting PrP using a molecule containing at least one positive charge and/or at least one glycosidic bond and a ligand other than a protein ligand

ABSTRACT

The present invention relates to a method for detecting PrP in a biological human or animal sample that may contain said PrP. The inventive method is characterized in that it uses a molecule containing at least one positive charge and/or at least one glycosidic bond and a ligand other than a protein ligand selected from macrocyclic ligands and glycosaminoglycans.

No figure